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In re Application of: **GARCIA-LADONA et al.**

Serial No.: **09/869,814**

Filing Date: **July 5, 2001**



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Attachments: **Substitute Brief on Appeal**
Claims Appendix, Evidence Appendix,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

IN RE APPLICATION

OF: GARCIA-LADONA

SERIAL NO. 09/869,814

FILED: JULY 05, 2001

FOR: BINDING PARTNERS FOR 5-HT₅ RECEPTORS FOR MIGRAINE TREATMENT

ATTY. DOCKET: 0480/01210

CONFIRMATION No.: 1323

GROUP ART UNIT: 1646

EXAMINER: D. JIANG

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SUBSTITUTEBRIEF ON APPEAL UNDER 37 C.F.R. §41.37

Sir:

This is a substitute Brief on Appeal regarding appellants' appeal from the Examiner's final rejection of Claims 29 to 32 and 34 to 36, dated February 25, 2005. Claims 29 to 32 and 34 to 36 are currently pending.

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REAL PARTY IN INTEREST:

The real party in interest is Abbott & Co. KG of Germany. An assignment document to record the change of ownership from Knoll Aktiengesellschaft to Abbott Laboratories GmbH is in preparation and will be filed in due course.

RELATED APPEALS AND INTERFERENCES:

To the best of appellants' knowledge and belief, there are no interferences or other appeals within the meaning of 37 CFR §41.37(c)(1)(ii).

STATUS OF THE CLAIMS:

Claims 29 to 32 and 34 to 36 are currently pending in the application. The current status of those claims is as follows: Claims 29 to 32 and 34 to 36 stand rejected.

STATUS OF THE AMENDMENTS:

New Claims 37 and 38 were presented by appellants in an amendment under 37 C.F.R. §1.116 dated July 14, 2004. In an Advisory action dated August 09, 2004, the Examiner indicated that the respective amendment would not be entered for purposes of appeal. No further amendments were filed in this application after final rejection.

The claims, therefore, stand as presented by appellants prior to the final action, i.e. as presented in appellants' reply dated November 20, 2003.

SUMMARY OF THE CLAIMED SUBJECT MATTER:

The invention disclosed and claimed by appellants relates, in its broadest aspect as set forth in Claim 29, to a method for treating migrainous cerebrovascular disorders which comprises administering to a subject in need thereof an effective amount of at least one binding partner for a 5-HT₅-receptor which exhibits a certain binding affinity and selectivity for the 5-HT₅-receptor over a 5-HT_{1D}-receptor.¹⁾

Binding partners according to appellants' invention are understood to be low molecular weight, usually synthetic compounds, antibodies or aptamers,²⁾ which bind to, or couple with, an

1) Cf., e.g., Claim 29; page 1, indicated lines 4 to 8, page 3, indicated lines 32 to 35, and page 4, indicated lines 1 to 27, of the application.

2) Cf., e.g., page 7, indicated lines 27 to 42, of the application.

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effector function of the receptor in a reversible or irreversible manner.³⁾ Hence, binding partners as referenced in applicants' claims can act as agonists, as antagonists, as partial agonists or as partial antagonists.⁴⁾

The migrainous cerebrovascular disorders which can be treated in accordance with appellants' method are in particular migraines,⁵⁾ and more specifically associated migraine, migraine equivalents, digestive migraine, ophthalmic migraine, ophthalmoplegic migraine, migraine rouge, cluster headache or cervical migraine,⁶⁾ and a particular embodiment of appellants' method is the acute treatment, i.e. the treatment when acute symptoms of migraine occur.⁷⁾

The binding selectivity for the 5-HT₅-receptor over the 5-HT_{1D}-receptor of the binding partners referenced in appellants' claims is such that

- the binding affinity for the 5-HT₅-receptor is at least 10 times greater than the binding affinity for a 5-HT_{1D}-receptor;
- the binding affinity for the 5-HT₅-receptor is at least 20 times greater than the binding affinity for a 5-HT_{1D}-receptor; or
- the binding affinity for the 5-HT₅-receptor is at least 50 times greater than the binding affinity for a 5-HT_{1D}-receptor,

respectively,⁸⁾ with the binding affinity of the binding partners for the 5-HT₅-receptor, as expressed by means of the inhibition constant K_i , preferably being less than 10^{-8} M.⁹⁾

The binding affinity of the binding partners referenced in appellants' claims is generally determined *in vitro* directly or using competition experiments,¹⁰⁾ and assays for the determination of binding affinities to 5-HT₅-receptors are in principle known in the art.¹¹⁾ The direct determination of binding affinities can be conducted calorimetrically, i.e. by measurement of the binding energy released.¹²⁾ Alternatively, the effectivity can be assessed qualitatively or quantitatively, *in vitro* and *in vivo*, by means of functional assays which are based on those effects which are caused when the binding partner binds to the 5-HT receptors in question. Such assessments are based, for instance, on the effects of the binding of GTP to G proteins on intercellular calcium levels, on phospholipase

3) Cf., e.g., page 6, indicated lines 23 and 24, page 3, indicated lines 27 to 45, of the application.

4) Cf., e.g., page 6, indicated lines 25 and 26, of the application.

5) Cf., e.g., Claim 34; page 14, indicated lines 11 and 12, of the application.

6) Cf., e.g., Claim 36; page 14, indicated lines 16 to 20, of the application.

7) Cf., e.g., Claim 35; page 14, indicated line 28, of the application.

8) Cf., e.g., Claims 29 to 31; page 4, indicated lines 12 to 15, and indicated lines 20 to 27, of the application.

9) Cf., e.g., Claim 32; page 5, indicated line 40, to page 6, indicated line 2, of the application.

10) Cf., e.g., page 5, indicated lines 40 to 42, page 7, indicated lines 44 to 46, and page 8, indicated lines 14 and 15, of the application.

11) Cf., e.g., page 7, indicated line 44, to page 8, indicated line 12, of the application.

12) Cf., e.g., page 8, indicated lines 14 to 18, of the application.

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C activity and/or on cAMP production.¹³⁾ The binding selectivity of a binding partner is determined by determining the binding affinity of the binding partner to each of the receptors in the same manner, and then comparing the values obtained in the respective investigations.¹⁴⁾

The asserted suitability of the binding partners for the treatment of migraine is preferably verified by animal models based on mechanisms connected to the formation of migraine,¹⁵⁾ i.e. based on

- protein extravasation;¹⁶⁾
- distribution of the carotid blood flow;¹⁷⁾
- expression and translocation of the nitroglycerin-induced c-fos gene;¹⁸⁾ and
- retinal and cortical spreading depression.¹⁹⁾

In contrast to the prior art which focuses on optimizing the selectivity of compounds for the 5-HT_{1D}-receptor in order to develop suitable treatments of migraine,²⁰⁾ appellants' invention provides for a migraine treatment which is conducted with binding partners which exhibit a selective binding affinity for the 5-HT_{1D}-receptor.²¹⁾

GROUND(S) OF REJECTION TO BE REVIEWED:

Whether the Examiner erred finding that appellants' Claims 29 to 32 and 34 to 36 were unpatentable under 35 U.S.C. §112, ¶1, for failing to meet the written description requirement and the enablement requirement.

ARGUMENT(S):

A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption.²²⁾ The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the

13) Cf. e.g., page 8, indicated line 26, to page 9, indicated line 21, of the application.

14) Cf., e.g., page 8, indicated lines 20 to 24, of the application.

15) Cf., e.g., page 11, indicated lines 27 to 30, and page 2, indicated lines 12 to 19, of the application.

16) Cf., e.g., page 11, indicated lines 32 to 44, of the application.

17) Cf., e.g., page 12, indicated lines 1 to 7, of the application.

18) Cf., e.g., page 12, indicated lines 9 to 15, of the application.

19) Cf., e.g., page 12, indicated lines 17 to 20, of the application.

20) Cf., e.g., page 3, indicated lines 12 to 21, of the application.

21) Cf., e.g., page 3, indicated lines 23 to 35, of the application.

22) See e.g., *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971).

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claims.²³⁾ In rejecting a claim, the examiner must set forth express findings of fact which support the lack of written description conclusion. These findings should:

- (A) Identify the claim limitation at issue; and
- (B) Establish a prima facie case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed.²⁴⁾

A publication of Read et al., *Migraine & Headache Pathophysiology*, "Cortical spreading depression and migraine," pp. 81-92, 1999, has been made of record by appellants. That publication showed that changes in metabolic, vascular and gene expression observed in experimental cortical spreading depression are complex and have a number of correlates in the clinic in migraine with or without aura.²⁵⁾

Retinal spreading depression is analogous to cortical spreading depression as stated in a declaration of Dr. Garcia-Ladona of record as illustrated by another publication of record.²⁶⁾

The examiner in the final rejection acknowledged that spreading depression is associated with migraine aura any may play a role in triggering classical migraine. Thus certain aspects of the publications and declaration made of record by appellants do not appear to require any further discussion.

The basis for the rejection is that only a single compound, HK 02-01, has been shown to meet the binding affinity terms of the appealed claims, and that the Garcia-Ladona declaration of record only relates to the relative efficacy of that compound compared to the prior art compound Sumatriptan. As support for this position, the examiner has relied on *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988) and *University of Rochester v. G.D. Searle + Co.*, 69 USPQ2d 1886 (Fed. Cir. 2004).

The Examiner seems to base the rejection at least partly on the assumption that the claimed method can be carried out only with either a 5-HT₅-receptor agonist or a 5-HT₅-receptor antagonist. The assumption is, however, not valid.

It is true that in principle one distinguishes between antagonist and agonistic effects that a drug may have on a particular receptor. However, this is only a rough classification. According to a more sophisticated (and realistic) view one has to differentiate between pure agonists, partial agonists, pure antagonists and partial antagonists (cf. the present specification on page 6, lines 23-26). To put it another way, any receptor agonist (perhaps with the exception of a 100% pure agonist) has a more or less pronounced antagonistic effect on the receptor. This is why one and the same drug may even

23) *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976).

24) Cf. MPEP §2163.

25) Cf. page 89 under "Conclusions."

26) Cf. DeLima et al., *Brain Research*, 614, pp. 45-51 (1993), addressed on page 12, indicated lines 17 to 20, of the application.

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elicit opposite effects depending on the state of the subject treated. For instance, dihydroergotamin may cause vasodilation in one patient and a vasoconstriction in another patient.

It should be emphasized in this connection that it is not required to know the effector function of the claimed 5-HT₅ binding partners in order to carry out the invention. For instance, the Garcia-Ladona declaration shows that all the skilled person has to do is carry out the *in vitro* screening process and test the thus identified compounds in an appropriate animal model.

The screening process is set forth in the present application and enables the skilled person to read out those compounds which have the required binding affinity for a 5-HT₅-receptor and selectivity over the 5-HT_{1D}-receptor. Appropriate animal tests are also set forth in the present application and enable the testing of the resulting compounds in terms of anti-migraine activity. All that is required for this screening and testing is routine experimentation. See *Wands, supra*.

Contrary to the Examiner's allegation, merely binding of a compound to a receptor can be used to predict the biological effect of the compound. In the present case, for instance, there is evidence that the binding affinity for 5-HT₅-receptor of a compound correlates with the efficacy of the treatment of migraine. That is to say, if one compares, for instance, the recommended therapeutic dose for known anti-migraine drugs with their binding affinity for 5-HT₅-receptor, it can be seen that the therapeutic dose increases as the binding affinity decreases:²⁷⁾

(R(+)-) Lisurid:	0.075 mg/day	8.2 10 ⁻⁹ M;
Dihydroergotamin:	3 mg/day	1.79 10 ⁻⁸ M;
Methylsergid:	2-6 mg/day	1.48 10 ⁻⁷ M;
Sumatriptan:	100 mg/day	7.6 10 ⁻⁶ M;

Please note that this comparison does not belong to the prior art but is based on the present invention.

In vitro and *in vivo* methods have been fully set out in the present specification as alluded to above, and it would not be difficult or require undue experimentation on the part of one skilled in the art to identify suitable compounds without the use of undue experimentation. This is set forth in the *Wands* decision cited by the examiner and in *In re Angstaadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (Fed. Cir. 1976). Also see MPEP §§2164.01 and 2164.06.

The claims in US 6,048,850, which was the subject of the *University of Rochester* case cited by the examiner, were drawn to a method of selectively inhibiting PGHS-2 activity by administering a compound that selectively inhibits activity of the PGHS-2 gene product. Thus, what was claimed was to use a compound having a certain effect in a method for achieving said effect. This is a circular statement.

²⁷⁾ The binding affinities are given in example 2, page 21, of the specification.

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In contrast, the claims in the present application are directed to a method for treating particular diseases, i.e., migrainous cerebrovascular diseases such as migraine. Thus the subject matter of the present claims is not simply confined to selectively inhibiting 5-HT₅-receptor activity by administering compounds that selectively inhibit the activity of the 5-HT₅-receptor. On the contrary, the present invention teaches for the first time that cerebrovascular disorders such as migraine can be effectively treated with binding partners for the 5-HT₅-receptor. Thus, it is the relationship between the 5-HT₅ binding affinity and the treatment of certain diseases which represents the contribution the present invention makes over the prior art.

The '850 patent, however, does not make such a contribution. Actually, the gist of the '850 patent is the cloning of the PGHS-2 gene and the provision of a screening method for identifying a compound that inhibits prostaglandin synthesis catalyzed by mammalian prostaglandin H synthase-2 (PGHS-2).

Two prior decisions cited in *University of Rochester* reflect the fact that physical properties can give a precise definition and that the principle set forth in *Metcalf* is still correct. They are *Eli Lilly & Co. v. Barr. Labs., Inc.*, 251 F.3d 955, 58 USPQ2d 1865 (Fed. Cir. 2001) and *Enzo Biochem., Inc. v. Gen-Probe, Inc.*, 323 F.3d 956, 63 USPQ 1609 (Fed. Cir. 2002). Indeed, as held in *Enzo*, disclosure of a nucleic acid can support a claim to nucleic acids that hybridize to it (i.e., that have a certain binding affinity for it).

The present specification sets forward several "animal models [based] on mechanisms which can underlie the formation of migraine" disorders.²⁸⁾ These animal models include protein extravasation, distribution of carotid blood flow, measurement of the nitroglycerin-induced c-fos gene expression and translocation, measurement of other transcription factors such as c-jun, zif268, or Homer gen isoforms, retinal spreading depression, and cortical spreading depression.²⁹⁾ These animal models are described in detail in the prior art, and testing identified 5-HT₅ binding partners using these models would be a matter of routine to the appropriately skilled artisan.

The specification contains assertions as to the efficacy of the 5-HT₅ binding partners in treating migraine disorders which must be taken as enabling in the absence of specific reasoning to the contrary. *In re Dinh-Nguyen*, 492 F.2d 856, 181 USPQ 46 (CCPA 1974); *In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971). The reasoning presented by the Examiner does not account for all information available to one skilled in the art, and is not, therefore, sufficient to overcome the established presumption.

The Garcia-Ladona declaration of record demonstrates the efficacy of 5-HT₅ binding partners of the appropriate binding affinities relative to 5-HT_{1D} affinity. The declaration supports the presumption that one of ordinary skill in the art would be able to carry out the presently claimed inven-

28) Cf. page 22, indicated lines 28 to 30, of the application.

29) Cf. page 7, indicated lines 15 to 21, and page 11, indicated line 32, to page 12, indicated line 20, of the application.

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tion based on the specification disclosure and knowledge already in the art. Although the present claims are drawn to a process, the decision *In re Bundy*, 642 F.2d 430, 434, 209 USPQ 48, 52 (Fed. Cir. 1981), is considered to be quite relevant to the present fact situation, especially the quotation which follows:

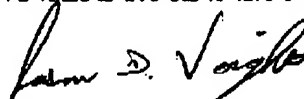
Early filing of an application with its disclosure of novel compounds which possess significant therapeutic use is to be encouraged. Requiring specific testing of the thousands of prostaglandin analogs encompassed by the present claim in order to satisfy the how-to-use requirement of §112 would delay disclosure and frustrate, rather than further, the interest of the public.

CONCLUSION

In light of the foregoing reasons and explanations, appellants respectfully urge that the Examiner's final rejection of appellants' Claims 29 to 32 and 34 to 36 under 35 U.S.C. §112, ¶1, as being unpatentable for failing to meet the written description requirement and the enablement requirement was in error. It is therefore respectfully requested that the Examiner's respective rejections be reversed. Favorable action is solicited.

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees, to Deposit Account No. 14.1437. Please credit any excess fees to such deposit account.

Respectfully submitted,
NOVAK DRUCE DELUCA & QUIGG



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Encl.: CLAIMS APPENDIX
EVIDENCE APPENDIX
RELATED PROCEEDINGS APPENDIX

JDV/BAS

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CLAIMS APPENDIX:

29. A method for treating migrainous cerebrovascular disorders which comprises administering to a subject in need thereof an effective amount of at least one binding partner for a 5-HT₅-receptor whose binding affinity for the 5-HT₅-receptor is at least 10 times greater than its binding affinity for a 5-HT_{1D}-receptor.
30. The method as claimed in claim 29, where the binding affinity of the binding partner for a 5-HT₅-receptor is at least 20 times greater than its binding affinity for a 5-HT_{1D}-receptor.
31. The method as claimed in claim 29, where the binding affinity of the binding partner for a 5-HT₅-receptor is at least 50 times greater than its binding affinity for a 5-HT_{1D}-receptor.
32. The method as claimed in claim 29, where the K_i value for binding of the binding partner to the 5-HT₅-receptor is less than 10⁻⁸ M.
34. The method as claimed in claim 29, wherein the migrainous cerebrovascular disorder is migraine.
35. The method as claimed in claim 34, wherein the binding partner is administered when acute symptoms of migraine occur.
36. The method as claimed in claim 34, wherein the migraine is a disorder selected from the group consisting of associated migraine, migraine equivalents, digestive migraine, ophthalmic migraine, ophthalmoplegic migraine, migraine rouge, cluster headache and cervical migraine.

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EVIDENCE APPENDIX:

Dr. Garcia-Ladona's Declaration dated November 17, 2003

Read et al., *Migraine & Headache Pathophysiology*, "Cortical spreading depression and migraine," pp. 81-92 (1999).

The papers were submitted with appellants' reply of November 20, 2003 (date of the Certificate of Mailing). Entry and consideration of the reply was acknowledged by the Examiner in the Office action of February 25, 2004. Copies of the respective papers are enclosed with this paper.

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RELATED PROCEEDINGS APPENDIX:

To the best of appellants' knowledge and belief, there are no interferences or other appeals within the meaning of 37 CFR §41.37(c)(1)(ii).

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Garcia-Ladona

Serial No. 09/869,814

Filed: 05/07/01

**FAXED**

Group/Art Unit: 1646

Examiner: JIANG, DONG

For : Binding partners for 5-HT5A receptors for the treatment of migraine

DECLARATION

1. I, Francisco Javier Garcia-Ladona, Ph.D., a citizen of Spain, hereby declare as follows:

I am a fully trained biologist. Having studied biology at the Universidad Autonoma of Barcelona, and graduating in Biology, I received from 1987 to 1992 a doctorate (Ph.D.) from the University Louis Pasteur Strasbourg in Molecular and Cell Biology specialty Neurochemistry. I have been employed by Abbott GmbH & Co. KG and formerly by Knoll Aktiengesellschaft of 67061 Ludwigshafen, Germany for 11 years as research scientist in the field of CNS disorders including neuropsychiatric diseases and neurodegeneration. I have been working in the field of serotonin receptors since 1992. I am therefore fully conversant with the prior art.

I am the inventor of the subject-matter disclosed and claimed in Appl. Ser. No. 09/869,814 and I am therefore familiar therewith.

2. I have read and fully understood the Office Action of May 22, 2003 and the references cited therein by the Examiner and conceived the experimental tests given in the specification of Appl. Ser. No. 09/869,814 or as described below.
3. Under my supervision, *in vitro* screening processes were performed in order to identify compounds which are suitable to treat cerebrovascular disorders such as migraine. Said processes comprised determining the binding affinity of candidate compounds for 5-HT5A and 5-HT1D receptors. In particular, those compounds whose binding affinity for 5-HT5A receptors is least 10-times higher than the binding affinity for 5-HT1D receptors were read out. *Inter alia* the following compound was identified having the binding affinities as indicated:

- HK02-01 ($K_i^{5-HT5A} = 3.3 \text{ nM}$; $K_i^{5-HT1D} > 1000 \text{ nM}$)

Thus, compound HK02-01 binds to the 5-HT5A receptor with an affinity that is more than 10-times higher than its affinity for the 5-HT1D receptor.

4. Sumatriptan is currently used in the treatment of migraine. For sumatriptan the binding affinity for the same receptors are as indicated:

- Sumatriptan ($K_i^{5-HT5A} > 1000 \text{ nM}$; $K_i^{5-HT1D} = 3 \text{ nM}$)

Thus, in contrast to the above compound HK02-01, sumatriptan binds to the 5-HT5A receptor with an affinity that is more than 10-times lower than its affinity for the 5-HT1D receptor.

5. Further, I evaluated whether the above compound HK02-01 is expected to be useful in a method for treating cerebrovascular disorders such as migraine. I asked Prof. Dr. Wolfgang Hanke at the Institute for Physiology of the University Hohenheim at 70593 Stuttgart, Germany, to investigate the effects of said compound in the retinal spreading depression.
6. Retinal spreading depression (rSD) is a well-recognized model for evaluating the efficacy of compounds in the treatment of migraine (see Fernandez de Lima V.M. et al. (1993) Brain Res. 614: 45-451; also referred to in the specification of Appl. Ser. No. 09/869,814).

Spreading Depression (SD) is an example of a physiological response of the neuronal tissue. It has been observed in all parts of the CNS, including the retina. Such SD waves after a proper stimulus (mechanical, chemical or electrical) propagate through the tissue with a velocity of $3-5 \text{ mm min}^{-1}$ and consist of a short period of hyper excitation, followed by a longer period of complete suppression of electrical activity of the tissue involved. The waves are accompanied by a variety of other changes, like potential changes, changes in ion homeostasis, changes in ion channel parameters and in cellular volume. The SD is a transient phenomenon; the tissue completely recovers after several minutes.

A compound that significantly decreases spreading velocity, is expected to be effective in the treatment of migraine.

7. Prof. Hanke determined spreading velocity as follows:

7.1 Chemicals

7.1.1 Ringer

Retinas were perfused with standard ringer solution of following composition:

100 mM	NaCl
6 mM	KCl
1 mM	MgSO ₄
1 mM	CaCl ₂ 2H ₂ O
1 mM	NaH ₂ PO ₄
30 mM	NaHCO ₃
10 mM	TRIS
30 mM	Glucose

All substances except CaCl₂ were dissolved in aqua bidest. The pH was adjusted with HCl to 7,5, then CaCl₂ was added and pH was adjusted to 7,4. The used salts were obtained in p.a. from Fluka, Merck and Sigma in Germany.

7.1.2 Applied test compounds

HK02-01 and sumatriptan were dissolved in 1 % DMSO (Dimethylsulfoxid). 1 % DMSO alone had no effect on the investigated parameters of the rSD.

7.2 Set up

The set up was mounted on vibration-damped table. It consisted of an aluminum plate with four hollows for the petri dishes, a heating pad, a perfusion system containing two four-channel peristaltic pumps with tube system and one movable camera, that was mounted on a motor driven carriage. The retinas were perfused with ringer solution with a constant rate of 1 ml/min.

All experiments were stored on video tapes for later evaluation of the different wave parameters with adequate software. The video equipment contained camera video recorder, monitor and video processor.

7.3 Preparation of the eye-cup

For the experiments, chicken in the age from 5 to 21 days were used. After decapitation the eyes were removed out of the eye socket. Eyes were sectioned close to the equator and vitreous body was removed with tweezers. The nosta-

in a petri dish and put into the set-up where they were perfused with ringer solution. Before the measurements started the retinas were allowed to recover for 30 min.

7.4 Protocol

For the velocity experiments several retinas were measured in parallel. The waves were elicited mechanically by gently touching the retina with a fine tungsten electrode. After each elicited wave the temperature was controlled. Then the camera was positioned over the next retina, where the next wave was elicited. After the same procedure has been carried out with all retinas, the camera was moved to the first retina again. After 30 minutes recovery a new measuring cycle started. The first two waves were taken as controls with standard ringer solution. Immediately after the second measuring cycle the perfusion solution was changed. The standard ringer solution was then replaced by ringer solution plus the test compound. Five cycles with test compound were measured followed by two cycles with standard ringer solution to see whether the effects were reversible.

7.5 Data evaluation

The velocity was measured by measuring the time the wave front needed to travel over a defined distance on the monitor. The data were calculated in mm/min. The means of the controls were taken as $y = 1$; means of wave velocity under the action of test compound were compared to the control.

Significance was calculated with adequate software (Graph Pd Prism, t-test).

8. Prof. Dr. Hanke reported the following results:

8.1 HK02-01:

The spreading velocity of the waves is summarized in the following data table (relative spreading velocity (y) for all concentrations at different times of stimulation). The value at $t = 0$ is the mean of the controls and set to $y = 1$. Values are given as means \pm SEM.

Time	10 μ M			30 μ M			45 μ M			60 μ M			75 μ M			100 μ M		
	y	SEM	n	y	SEM	n	y	SEM	n	y	SEM	n	y	SEM	n	y	SEM	n
00	1,00	0,0	8	1,00	0,00	4	1,00	0,00	4	1,00	0,00	4	1,00	0,00	1	1,00	0,00	4
30	1,06	0,04	8	1,10	0,04	4	0,99	0,06	4	0,98	0,03	4	0,99	0,00	1	0,98	0,14	4
60	0,99	0,04	8	1,08	0,04	4	0,91	0,03	4	0,88	0,04	4	0,80	0,00	1	0,80	0,11	4
90	0,98	0,04	8	1,04	0,03	4	0,86	0,05	4	0,82	0,04	4	0,75	0,00	1	0,27	0,16	4
120	0,98	0,04	9	1,00	0,04	4	0,86	0,05	4	0,78	0,03	4	0,71	0,00	1	0,14	0,14	4

HK02-01 significantly decreases spreading velocity. As can be seen, the higher the concentration of HK02-01 the more effectively the spreading velocity is decreased. HK02-01 is thus be expected to be useful for treating migraine.

8.2 Sumatriptan:

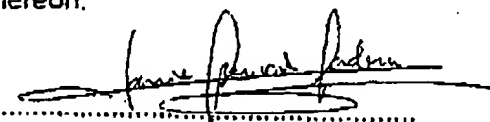
The spreading velocity of the waves is summarized in the following data table (relative spreading velocity (y) for all concentrations at different times of stimulation). The value at $t = 0$ is the mean of the controls and set to $y = 1$. Values are given as means \pm SEM.

Time	400 μ M			500 μ M			800 μ M			1 mM			1.5 mM		
	y	SEM	n	y	SEM	n	y	SEM	n	y	SEM	n	y	SEM	n
00	1,00	0,0	2	1,00	0,00	2	1,00	0,00	2	1,00	0,00	4	1,00	0,00	3
20	0,92	0,02	2	0,97	0,03	2	0,85	0,00	1	0,77	0,07	4	0,67	0,02	3
40	0,93	0,01	2	0,88	0,00	1	0,81	0,02	2	0,70	0,06	4	0,48	0,08	3
60	0,93	0,01	2	0,88	0,00	1	0,83	0,00	2	0,69	0,04	4	0,54	0,02	3
80	0,95	0,01	2	0,88	0,00	2	0,82	0,01	2	0,69	0,02	4	0,52	0,04	2
100	0,98	0,02	2	-	-	-	0,84	0,08	2	-	-	4	-	-	-

Sumatriptan significantly decreases spreading velocity. As can be seen, the higher the concentration of sumatriptan the more effectively the spreading velocity is decreased. This confirms that sumatriptan is useful for treating migraine.

9. Further, from the above results it can also be seen that the compound having a higher binding affinity for the 5-HT_{5A} receptor than for the 5-HT_{1D} receptor (i.e. HK02-01) decreases spreading velocity more significantly than the compound having a lower binding affinity for the 5-HT_{5A} receptor than for the 5-HT_{1D} receptor (i.e. sumatriptan). This shows that migraine is associated with the 5-HT_{5A} receptor. Moreover, since sumatriptan is known to be effective in the treatment of migraine, it is thus reasonable to expect that compounds having a higher binding affinity for the 5-HT_{5A} receptor than for the 5-HT_{1D} receptor (such as HK02-01) be even more effective in the treatment of migraine.
10. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine of imprisonment, or both, under section 1001 of title 18 of the U.S. code and that such willful false statements may jeopardize the validity of the above-identified application or patent issuing thereon.

Ludwigshafen, 17.11.2003



Comorbidity of depression and migraine

INTRODUCTION

Depression and migraine are common disorders that often co-occur. The prevalence of depression in migraineurs is estimated to be between 10% and 30%, while the prevalence of migraine in depressed patients is estimated to be between 10% and 20%. The comorbidity of these two disorders has been the subject of extensive research, and it is now clear that the two disorders are not simply associated, but rather, they are interrelated. This paper will review the current literature on the comorbidity of depression and migraine, focusing on the clinical, genetic, and neurobiological aspects of the relationship.

ANIMAL MODEL

The animal model of depression and migraine is based on the concept of a "stressor" that triggers the onset of both disorders. In this model, a stressor (such as a change in environment, a social conflict, or a physical injury) leads to a cascade of physiological and biochemical changes that ultimately result in the development of both depression and migraine. This model is supported by a number of studies in which stressors have been shown to increase the risk of both disorders in animals. For example, in a study by [Author], rats that were exposed to a chronic stressor (such as a constant light/dark cycle) developed both depressive-like behaviors and migraine-like symptoms. Similarly, in a study by [Author], mice that were exposed to a social stressor (such as a change in social hierarchy) developed both depressive-like behaviors and migraine-like symptoms.

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of CSD can be induced following application of a chemical stimulus. In the model, the effect of prolonged contact resulting in a significant change in put away chemical after per measurement, which indicates an effect of an independent body.

The reaction of the system to the effect of CSD can also be induced following application of a chemical stimulus. In the model, the effect of prolonged contact resulting in a significant change in put away chemical after per measurement, which indicates an effect of an independent body.

These experiments should be used to assess the amount of time of the CSD in the system. In the model, the effect of prolonged contact resulting in a significant change in put away chemical after per measurement, which indicates an effect of an independent body.

study in spontaneous migraine. RTI increases in cortical bloodflow and regions of the thalamus were observed during migraine and increases of this attack with unimpaired normalized regional cerebral bloodflow changes in the cortex but had no effect on the changes in bloodflow in the brainstem. These studies have been taken to demonstrate the presence of a 'generator' region for migraine and provide evidence for the symptomatic effects of parasympathetic therapy.

However, a number of issues need to be considered when interpreting regional cerebral bloodflow changes to migraine. Local bloodflow will increase in regions where metabolic demand is increased. During a spontaneous or provoked migraine attack, the net response will depend on the integration of pathways activated during the migraine process and pathways activated because of the migraine process. Pain, nausea, anxiety and 'functional well-being' may all provide activation of specific CNS areas which complicate interpretation of data.

Collectively, recent evidence supports the concept of a spreading oligogenic occurrence in migraine patients, which is consistent with the effects of spreading depression in experimental models. Magnetic resonance imaging (MRI) of the brain during CSD by Gardner-Medwin et al (1991) using a T₂/gradient echo protocol showed increases of 15% in signal intensity during the passage of a CSD wave. This increase in signal intensity could be attributed to increased tissue bloodflow and consequent increases in oxygen coefficients of relaxation. Elegant studies of Gao et al (1990) which used functional MRI with blood oxygen level dependent contrast (BOLD) imaging to study occipital cortex function during visually evoked bursts in migraine patients, detected a spreading neuromolecular 'wave' consistent with CSD in both migraine with and without aura

CSD and migraine

Measurement of circulating blood levels of several neurotransmitters (GABA, 5HT, histamine, noradrenaline, dopamine, norepinephrine and acetylcholine) and NO release during CSD. The levels of these neurotransmitters and NO release during CSD have been shown to increase in various concentrations of cortical and subcortical regions. CSD induces a reduction in the levels of GABA, 5HT, histamine, noradrenaline, dopamine, norepinephrine and acetylcholine in the cortex and subcortical regions. CSD also induces a reduction in the levels of NO release in the cortex and subcortical regions.

These changes in neurotransmitter levels during CSD are consistent with the changes in neurotransmitter levels during migraine. CSD also induces a reduction in the levels of GABA, 5HT, histamine, noradrenaline, dopamine, norepinephrine and acetylcholine in the cortex and subcortical regions. CSD also induces a reduction in the levels of NO release in the cortex and subcortical regions. These changes in neurotransmitter levels during CSD are consistent with the changes in neurotransmitter levels during migraine.

These changes in neurotransmitter levels during CSD are consistent with the changes in neurotransmitter levels during migraine.

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These changes in neurotransmitter levels during CSD are consistent with the changes in neurotransmitter levels during migraine.

Fig. 2.1. Microscopic views of white matter.

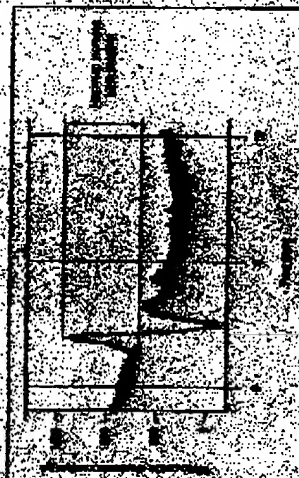


Table 2.1. Microscopic views of white matter showing axons and myelin sheath.

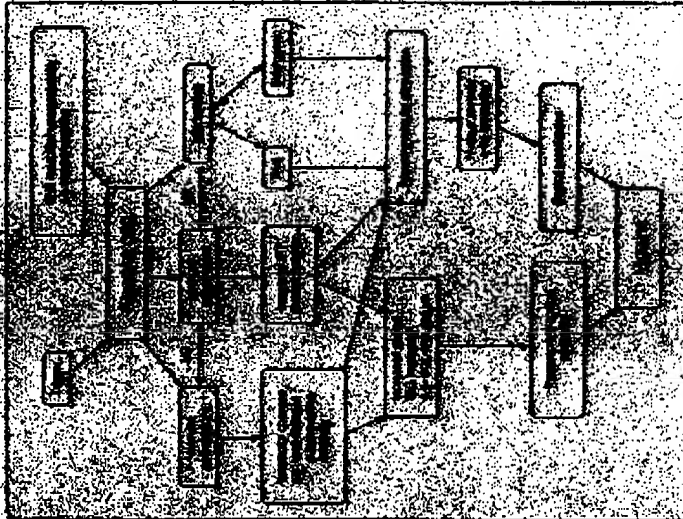
Structure	Microscopic views	Fig. 2.1
Cat	Cat	Fig. 2.1
Cat	Cat	Fig. 2.1
Human	Human	Fig. 2.1
Human	Human	Fig. 2.1
Human	Human	Fig. 2.1
Human	Human	Fig. 2.1

induce expression of nNOS in astrocytes during reactive gliosis at 4 hours post-GSD induction. The increased expression of other proteins involved in the inflammatory cascade have also been noted. Changes in *Cor2* mRNA expression have also been shown to occur following amyloid deposition [56].

TRIGGERING OF CSD

The initiation of CSD activity in the supraspinal cortex requires a number of factors. Overmeyer and Ulfhake¹⁰ have proposed that CSD is initiated through an extracellular accumulation of K^+ , as the initial event, leading to an increase in presynaptic glutamate and the release of Mg^{2+} blocks from NMDA channels on the postsynaptic membranes. The authors note that while initiation of glutamate neurotransmission is a prerequisite for CSD initiation, other processes may also be involved such as gap junction activity. The mechanism of superspreading¹¹ or homeostasis in this model has yet to be addressed. Extracellular K^+ released in the brain is primarily by glial cells and inactive synaptic release of several neurotransmitters, including intracellular accumulation of K^+ during postsynaptic Ca^{2+} ATPase formation in active Na^+ ATPase transporters and extracellular diffusion of extracellular K^+ migration.

Studies by Rand et al. demonstrate that furosemide pretreatment in a rat model of K^+ -attributed CSD inhibited CSD generation. The mechanism of inhibition of spontaneous CSD activated by furosemide is unknown, it may represent a desensitization in K^+ drive, or equally non-specific effects of furosemide such as inhibition of

[illegible][illegible]

Ex 4: The CD sentence

STOBILOTYPE

It is common to regard the *Salmonella* flagellin as a protein of low molecular weight. However, it is important to realize that the flagellin of *Salmonella* is a very large protein, with a molecular weight of 50,000. The flagellin of *Salmonella* is a very large protein, with a molecular weight of 50,000. The flagellin of *Salmonella* is a very large protein, with a molecular weight of 50,000.

For example, if you're looking for a new car, you might want to consider the following factors:

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Neurotoxicity in Inflammation

Neurotoxicity in Inflammation

Neurotoxicity in Inflammation

NEUROTOXICITY

The central nervous system is highly sensitive to various types of injury and damage. Neurotoxicity is a general term used to describe any type of damage to the nervous system. It can be caused by a variety of factors, including physical trauma, chemical exposure, infection, and autoimmune disease. Neurotoxicity can affect any part of the nervous system, including the brain, spinal cord, and peripheral nerves. The symptoms of neurotoxicity can vary widely, depending on the location and extent of the damage. Common symptoms include weakness, numbness, tingling, and pain. In severe cases, neurotoxicity can lead to paralysis and death.

Neurotoxicity is a complex phenomenon that involves a variety of factors. One of the most common causes of neurotoxicity is physical trauma, such as a head injury or a spinal cord injury. Trauma can damage the nervous system by tearing or crushing the nerves. Chemical exposure is another common cause of neurotoxicity. Many chemicals, including pesticides, herbicides, and industrial solvents, can be neurotoxic. Infection is also a common cause of neurotoxicity. Bacteria, viruses, and fungi can all damage the nervous system. Autoimmune disease is a less common cause of neurotoxicity. In autoimmune disease, the body's immune system attacks the nervous system, causing damage. Neurotoxicity can be a serious and potentially life-threatening condition. It is important to seek medical attention if you experience any symptoms of neurotoxicity. Early diagnosis and treatment can help to minimize the damage and improve the outcome.

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